FIGURE 1

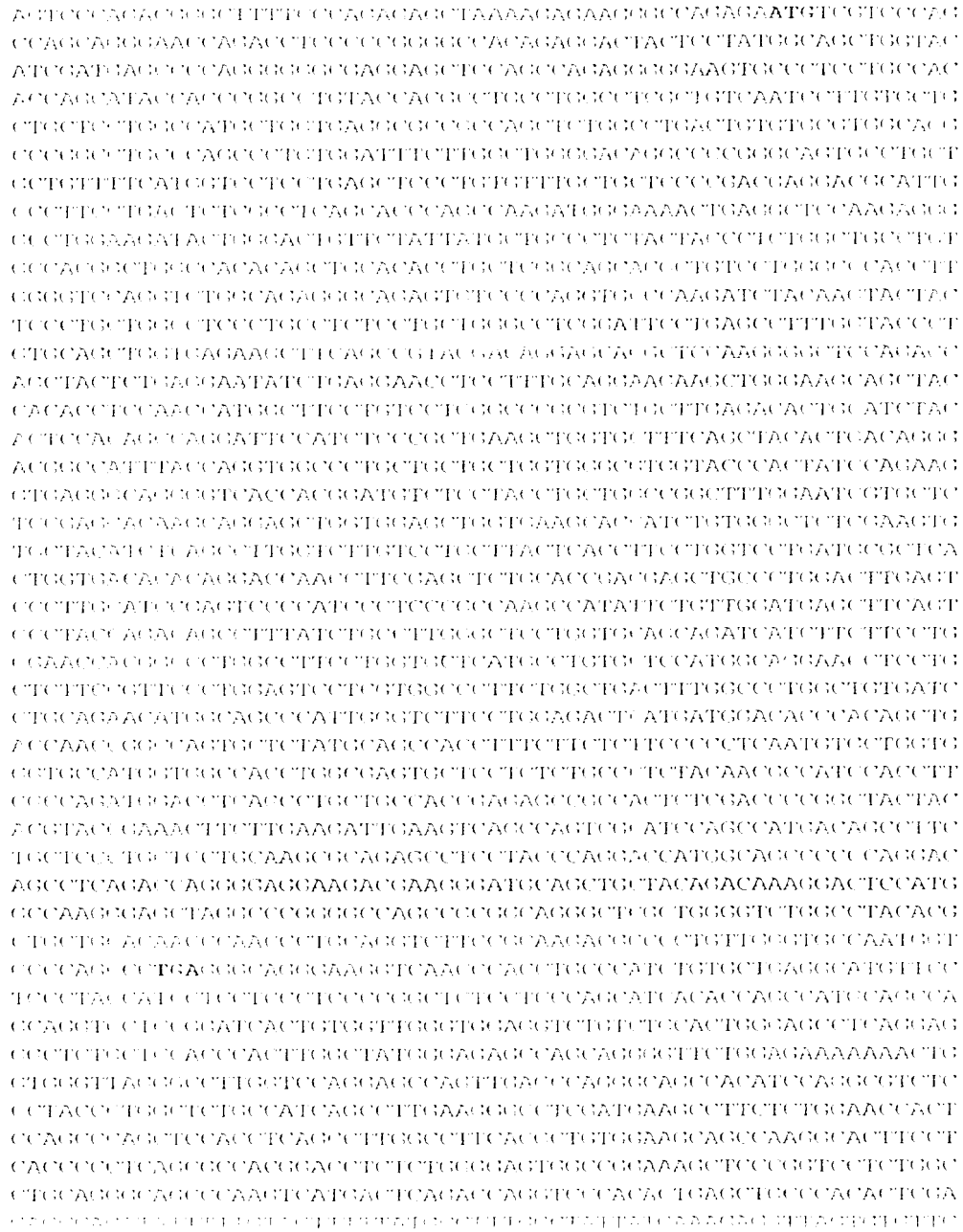


FIGURE 2

MSSQFAGNQTSPGATEDYSYGSWYIDEPQGGEELQPEGEVPSCHTSIPPGLYHACLASLS
ILVLLLLAMLVRRRQLWPDCVRGRPGLPSPVDFLAGDRPRAVPAAVFMVLLSSLCLLLPD
EDALPFLTLASAPSQDGKTEAPRGAWKILGLFYYAALYYPLAACATAGHTAAHLLGSTLS
WAHLGVQVWQRAECPQVPKIYKYYSLLASLPLLLGLGFLSLWYPVQLVRSFSRRTGAGSK
GLQSSYSEEYLRNLLCRKKLGSSYHTSKHGFLSWARVCLRHCIYTPQPGFHLPLKLVLSA
TLTGTAIYQVALLLLVGVVPTIQKVRAGVTTDVSYLLAGFGIVLSEDKQEVVELVKHHLW
ALEVCYISALVLSCLLTFLVLMRSLVTHRTNLRALHRGAALDLSPLHRSPHPSRQAIFCW
MSFSAYQTAFICLGLLVQQIIFFLGTTALAFLVLMPVLHGRNLLLFRSLESSWPFWLTLA
LAVILQNMAAHWVFLETHDGHPQLTNRRVLYAATFLLFPLNVLVGAMVATWRVLLSALYN
AIHLGQMDLSLLPPRAATLDPGYYTYRNFLKIEVSQSHPAMTAFCSLLLQAQSLLPRTMA
APQDSLRPGEEDEGMQLLQTKDSMAKGARPGASRGRARWGLAYTLLHNPTLQVFRKTALL
GANGAQP

Important features of the protein:
Signal peptide:

None

Transmembrane domain:

54-69
102-119
148-166
207-222
301-320
364-380
431-451
474-489
560-535

Motif file:
Motif name: N-glycosylation site.

8-12

Motif name: N-myristoylation site.

50-56
176-182
241-247
317-323
341-347
525-531
627-633
631-637
640-646
661-667

[illegible] attachment site

Motif name: [illegible]

132-140

FIGURE 3A

PRO	XXXXXXXXXXXXXXX	(Length = 15 amino acids)
Comparison Protein	XXXXXYYYYYYY	(Length = 12 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide)

5 divided by 15 = 33.3%

FIGURE 3B

PRO	XXXXXXXXXX	(Length = 10 amino acids)
Comparison Protein	XXXXXYYYYYYZZYZ	(Length = 15 amino acids)

% amino acid sequence identity

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide)

5 divided by 10 = 50%

FIGURE 3C

PRO DNA NNNNNNNNNNNNN (Length 14 nucleotides)

Comparison DNA NNNNNNLLLLLLLLL (Length 16 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO DNA nucleic acid sequence)

6 divided by 14 = 42.9%

FIGURE 3D

PRO DNA NNNNNNNNNNNN (Length = 12 nucleotides)

Comparison DNA NNNNI LLVV (Length = 9 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN 2) divided by (the total number of nucleotides of the PRO DNA nucleic acid sequence)

4 divided by 12 = 33.3%

FIGURE 4A

```
/*
 *
 * C C increased from 12 to 15
 * Z is average of EQ
 * B is average of ND
 * match with stop is  M, stop stop    0, J (joker) match    0
 */
#define    M        8        /* value of a match with a stop */

int        day[26][26]    {
/*      A  B  C  D  E  F  G  H  I  J  K  L  M  N  O  P  Q  R  S  T  U  V  W  X  Y  Z */
/* A */    { 2, 0, 2, 0, 0, 4, 1, 1, 1, 0,-1,-2,-1, 0, M, 1, 0, 2, 1, 1, 0, 0, 6, 0, 3, 0},
/* B */    { 0, 3, 4, 3, 2,-5, 0, 1, 2, 0, 0, 3, 2, 2, M, 1, 1, 0, 0, 0, 0, 2,-5, 0, 3, 1},
/* C */    { 2, 4,15, 5, 5,-4, 3, 3, 2, 0, 5, 6, 5, 4, M, 3, 5, 4, 0, 2, 0, 2, 8, 0, 0, 5},
/* D */    { 0, 3, 5, 4, 3, 6, 1, 1, 2, 0, 0, 4, 3, 2, M, 1, 2, 1, 0, 0, 0, 2,-7, 0, 4, 2},
/* E */    { 0, 2, 5, 3, 4, 5, 0, 1, 2, 0, 0, 3, 2, 1, M, 1, 2, 1, 0, 0, 0, 2, 7, 0, 4, 3},
/* F */    { 4, 5, 4, 6, 5, 9, 5, 2, 1, 0,-5, 2, 0, 4, M, 5, 5, 4, 3, 3, 0, 1, 0, 0, 7, 5},
/* G */    { 1, 0, 3, 1, 0, 5, 5, 2, 3, 0, 2, 4, 3, 0, M, 1, 1, 3, 1, 0, 0, 1, 7, 0, 5, 0},
/* H */    { 1, 1, 3, 1, 1, 2, 2, 6, 2, 6, 0, 2, 2, 2, M, 0, 3, 2, 1, 1, 0, 2, 3, 0, 0, 2},
/* I */    { 1, 2, 2, 2, 2, 1, 3, 2, 5, 0, 2, 2, 2, 2, M, 2, 2, 2, 1, 0, 0, 4, 5, 0, 1, 2},
/* J */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */    { 1, 0, 5, 0, 0, 5, 2, 0, 2, 0, 5, 3, 0, 1, M, 1, 1, 3, 0, 0, 0, 2, 3, 0, 4, 0},
/* L */    { 2, 3, 6, 4, 3, 2, 4, 2, 2, 0, 3, 6, 4, 3, M, 3, 2, 3, 3, 1, 0, 2, 2, 0, 1, 2},
/* M */    { 1, 2, 5, 3, 2, 0, 3, 2, 2, 0, 0, 4, 6, 2, M, 2, 1, 0, 2, 1, 0, 2, 4, 0, 2, 1},
/* N */    { 0, 2, 4, 2, 1, 4, 0, 2, 2, 0, 1, 3, 2, 2, M, 1, 1, 0, 1, 0, 0, 2, 4, 0, 2, 1},
/* O */                                                  { M, M, M, M, M, M, M, M, M, M, M, M, M, M,
0, M, M, M, M, M, M, M, M, M, M, M, M},
/* P */    { 1, 1, 3, 1, 1, 5, 1, 0, 2, 0, 1, 3, 2, 1, M, 6, 0, 0, 1, 0, 0, 1, 6, 0, 5, 0},
/* Q */    { 0, 1, 5, 2, 2, 5, 1, 3, 2, 0, 1, 2, 1, 1, M, 0, 4, 1, 1, 1, 0, 2, 5, 0, 4, 3},
/* R */    { 2, 0, 4, 1, 1, 4, 3, 2, 2, 0, 3, 3, 0, 0, M, 0, 1, 6, 0, 1, 0, 2, 2, 0, 4, 0},
/* S */    { 1, 0, 0, 0, 0, 3, 1, 1, 1, 0, 0, 3, 2, 1, M, 1, 1, 0, 2, 1, 0, 1, 2, 0, 3, 0},
/* T */    { 1, 0, 2, 0, 0, 3, 0, 1, 0, 0, 0, 1, 1, 0, M, 0, 1, 1, 1, 3, 0, 0, 5, 0, 3, 0},
/* U */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */    { 0, 2, 2, 2, 2, 1, 1, 2, 4, 0, 2, 2, 2, 2, M, 1, 2, 2, 1, 0, 0, 4, 6, 0, 2,-2},
/* W */    { 6, 5, 8, 7, 7, 0, 7, 3, 5, 0, 3, 2, 4,-4, M, 6, 5, 2, 2, 5, 0, 6,17, 0, 0, 6},
/* X */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */    { 3, 3, 0, 4, 4, 7, 5, 0, 1, 0,-1, 1, 2,-2, M, 5, 4, 4, 3, 3, 0, 2, 0, 0,10, 4},
/* Z */    { 0, 1, 5, 2, 3, 5, 0, 2, 2, 0, 0, 2, 1, 1, M, 0, 3, 0, 0, 0, 0, 0, 2, 6, 0, 4, 4}
};
```

FIGURE 4B

```
/*
 */
#include <stdio.h>
#include <ctype.h>

#define  MAXJMP     16      /* max jumps in a diag */
#define  MAXGAP     24      /* don't continue to penalize gaps larger than this */
#define  JMPS       1024    /* max jmps in an path */
#define  MX         4       /* save if there's at least MX-1 bases since last jmp */

#define  DMAT       3       /* value of matching bases */
#define  DMIS       0       /* penalty for mismatched bases */
#define  DINS0      8       /* penalty for a gap */
#define  DINS1      1       /* penalty per base */
#define  PINS0      8       /* penalty for a gap */
#define  PINS1      4       /* penalty per residue */

struct jmp {
        short           n[MAXJMP];      /* size of jmp (neg for dely) */
        unsigned short  x[MAXJMP];      /* base no. of jmp in seq x */
};                                      /* limits seq to 2^16-1 */

struct diag {
        int             score;          /* score at last jmp */
        long            offset;         /* offset of prev block */
        short           jmp;            /* current jmp index */
        struct jmp      jp;             /* list of jmps */
};

struct path {
        int     spc;            /* number of leading spaces */
        short   n[JMPS];/* size of jmp (gap) */
        int     x[JMPS];/* loc of jmp (last elem before gap) */
};

char            *ofile;                 /* output file name */
char            *namex[2];              /* seq names: getseqs() */
char            *prog;                  /* prog name for err msgs */
char            *seqx[2];               /* seqs: getseqs() */
int             dmax;                   /* best diag: nw() */
int             dmax0;                  /* final diag */
int             dna;                    /* set if dna: main() */
int             endgaps;                /* set if penalizing end gaps */
int             gapx, gapy;             /* total gaps in seqs */
int             len0, len1;             /* seq lens */
int             ngapx, ngapy;           /* total size of gaps */
int             smax;                   /* max score: nw() */
int             *xbm;                   /* bitmap for matching */
long            offset;                 /* current offset in jmp file */
struct  diag    *dx;                    /* holds diagonals */
struct  path    pp[2];                  /* holds path for seqs */

char            *calloc(), *malloc(), *index(), *strcpy();
```

FIGURE 4C

```
/* Needleman Wunsch alignment program
 *
 * usage: progs file1 file2
 *   where file1 and file2 are two dna or two protein sequences.
 *   The sequences can be in upper- or lower-case an may contain ambiguity
 *   Any lines beginning with ';', '>' or '<' are ignored
 *   Max file length is 65535 (limited by unsigned short x in the jmp struct)
 *   A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
 *   Output is in the file "align.out"
 *
 * The program may create a tmp file in /tmp to hold info about traceback.
 * Original version developed under BSD 4.3 on a vax 8650.
 */
#include "nw.h"
#include "day.h"

static    dbval[26] = {
        1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static    pbval[26] = {
        1, 2|(1<<('D'-'A')), (1<<('C'-'A')), 4, 8, 16, 32, 64,
        128, 256, 0xFFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
        1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
        1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)                                                    main
int     ac;
char    *av[];
{
        prog = av[0];
        if (ac != 3) {
                fprintf(stderr,"usage: %s file1 file2\n", prog);
                fprintf(stderr,"where file1 and file2 are two dna or two protein sequences.\n");
                fprintf(stderr,"The sequences can be in upper- or lower-case\n");
                fprintf(stderr,"Any lines beginning with ';' or '>' are ignored\n");
                fprintf(stderr,"Output is in the file \"align.out\"\n");
                exit(1);
        }
        namex[0] = av[1];
        namex[1] = av[2];
        seqx[0] = getseq(namex[0], &len0);
        seqx[1] = getseq(namex[1], &len1);
        xbm = (dna)? dbval : pbval;

        endgaps = 0;                    /* 1 to penalize endgaps */
        ofile = "align.out";            /* output file */

        nw();                   /* fill in the matrix, get the possible jmps */
        readjmps();             /* get the actual jmps */
        print();                /* print stats, alignment */
```

FIGURE 4D

```
/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
 * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y
 */
nw()                                                                    nw
{
        char            *px, *py;       /* seqs and ptrs */
        int             *ndely, *dely;  /* keep track of dely */
        int             ndelx, delx;    /* keep track of delx */
        int             *tmp;           /* for swapping row0, row1 */
        int             mis;            /* score for each type */
        int             ins0, ins1;     /* insertion penalties */
        register        id;             /* diagonal index */
        register        ij;             /* jmp index */
        register        *col0, *col1;   /* score for curr, last row */
        register        xx, yy;         /* index into seqs */

        dx = (struct diag *)g_calloc("to get diags", len0 + len1 + 1, sizeof(struct diag));

        ndely = (int *)g_calloc("to get ndely", len1 + 1, sizeof(int));
        dely = (int *)g_calloc("to get dely", len1 + 1, sizeof(int));
        col0 = (int *)g_calloc("to get col0", len1 + 1, sizeof(int));
        col1 = (int *)g_calloc("to get col1", len1 + 1, sizeof(int));
        ins0 = (dna)? DINS0 : PINS0;
        ins1 = (dna)? DINS1 : PINS1;

        smax = -10000;
        if (endgaps) {
                for (col0[0] = dely[0] = -ins0, yy = 1; yy < len1; yy++) {
                        col0[yy] = dely[yy] = col0[yy-1] - ins1;
                        ndely[yy] = yy;
                }
                col0[0] = 0;            /* Waterman Bull Math Biol 84 */
        }
        else
                for (yy = 1; yy <= len1; yy++)
                        dely[yy] = -ins0;

        /* fill in match matrix
         */
        for (px = seqx[0], xx = 1; xx < len0; px++, xx++) {
                /* initialize first entry in col
                 */
                if (endgaps) {
                        if (xx == 1)
                                col1[0] = delx = -(ins0+ins1);
                        else
                                col1[0] = delx = col0[0] - ins1;
                        ndelx = xx;
                }
```

FIGURE 4E


```
for (py = seqx[1], yy = 1; yy < len1; py++, yy++) {
        mis = col0[yy-1];
        if (dna)
                mis += (xbm['px-'A'] & xbm['py-'A'])? DMAT : DMIS;
        else
                mis += dav['px-'A']['py-'A'];

        /* update penalty for del in x seq;
         * favor new del over ongong del
         * ignore MAXGAP if weighting endgaps
         */
        if (endgaps || ndely[yy] < MAXGAP) {
                if (col0[yy] - ms0 < dely[yy]) {
                        dely[yy] = col0[yy] - (ms0+ms1);
                        ndely[yy] = 1;
                } else {
                        dely[yy] += ms1;
                        ndely[yy]++;
                }
        } else {
                if (col0[yy] - (ms0+ms1) < dely[yy]) {
                        dely[yy] = col0[yy] - (ms0+ms1);
                        ndely[yy] = 1;
                } else
                        ndely[yy]++;
        }

        /* update penalty for del in y seq;
         * favor new del over ongong del
         */
        if (endgaps || ndelx < MAXGAP) {
                if (col1[yy-1] - ms0 < delx) {
                        delx = col1[yy-1] - (ms0+ms1);
                        ndelx = 1;
                } else {
                        delx += ms1;
                        ndelx++;
                }
        } else {
                if (col1[yy-1] - (ms0+ms1) < delx) {
                        delx = col1[yy-1] - (ms0+ms1);
                        ndelx = 1;
                } else
                        ndelx++;
        }

        /* pick the maximum score; we're favoring
         * mis over any del and delx over dely
         */
```

FIGURE 4F


```
                        id = xx - yy + len1 - 1;
                        if (mns > ndlx && mns > ndlv[yy])
                                coll[yy] = mns;
                        else if (ndlx > ndlv[yy]) {
                                coll[yy] = ndlx;
                                n = dx[id].njmp;
                                if (dx[id].jp.n[0] && (!dna || (ndlx > MAXJMP
                                && xx > dx[id].jp.x[n] + MX) || mns > dx[id].score + DINS0)) {
                                        dx[id].njmp++;
                                        if (++n > MAXJMP) {
                                                writejmps(id);
                                                n = dx[id].njmp = 0;
                                                dx[id].offset = offset;
                                                offset += sizeof(struct jmp) + sizeof(offset);
                                        }
                                }
                                dx[id].jp.n[n] = ndlx;
                                dx[id].jp.x[n] = xx;
                                dx[id].score = ndlx;
                        }
                        else {
                                coll[yy] = ndlv[yy];
                                n = dx[id].njmp;
                                if (dx[id].jp.n[0] && (!dna || (ndlv[yy] > MAXJMP
                                && xx > dx[id].jp.x[n] + MX) || mns > dx[id].score + DINS0)) {
                                        dx[id].njmp++;
                                        if (++n > MAXJMP) {
                                                writejmps(id);
                                                n = dx[id].njmp = 0;
                                                dx[id].offset = offset;
                                                offset += sizeof(struct jmp) + sizeof(offset);
                                        }
                                }
                                dx[id].jp.n[n] = ndlv[yy];
                                dx[id].jp.x[n] = xx;
                                dx[id].score = ndlv[yy];
                        }
                        if (xx == len0 && yy < len1) {
                                /* last col
                                 */
                                if (endgaps)
                                        coll[yy] = ms0 + ms1*(len1 - yy);
                                if (coll[yy] > smax) {
                                        smax = coll[yy];
                                        dmax = id;
                                }
                        }
                }
                if (endgaps && xx < len0)
                        coll[yy-1] = ms0 + ms1*(len0 - xx);
                if (coll[yy-1] > smax) {
                        smax = coll[yy-1];

        (void) free((char *) ndlv);
        (void) free((char *)dlv);
        (void) free((char *)col0); (void) free((char *)coll); }
```

FIGURE 4G

```
/*
 *
 * print()  - only routine visible outside this module
 *
 * static:
 * getmat()  - trace back best path, count matches: print()
 * pr_align()  - print alignment of described in array p[]: print()
 * dumpblock()  - dump a block of lines with numbers, stars: pr_align()
 * nums()  - put out a number line: dumpblock()
 * putline()  - put out a line (name, [num], seq, [num]): dumpblock()
 * stars()  - put a line of stars: dumpblock()
 * stripname() - strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC     3
#define P_LINE  256     /* maximum output line */
#define P_SPC   3       /* space between name or num and seq */

extern  day[26][26];
int     olen;           /* set output line length */
FILE    *fx;            /* output file */

print()                                                          print
{
        int     lx, ly, firstgap, lastgap;      /* overlap */

        if ((fx = fopen(ofile, "w")) == 0) {
                fprintf(stderr,"%s: can't write %s\n", prog, ofile);
                cleanup(1);
        }
        fprintf(fx, "< first sequence: %s (length = %d)\n", namex[0], len0);
        fprintf(fx, "> second sequence: %s (length = %d)\n", namex[1], len1);
        olen = 60;
        lx = len0;
        ly = len1;
        firstgap = lastgap = 0;
        if (dmax < len1 - 1) {          /* leading gap in x */
                pp[0].spc = firstgap = len1 - dmax - 1;
                ly -= pp[0].spc;
        }
        else if (dmax > len1 - 1) {     /* leading gap in y */
                pp[1].spc = firstgap = dmax - (len1 - 1);
                lx -= pp[1].spc;
        }
        if (dmax0 < len0 - 1) {         /* trailing gap in x */
                lastgap = len0 - dmax0 - 1;
                lx -= lastgap;
        }
        else if (dmax0 > len0 - 1) {    /* trailing gap in y */
                lastgap = dmax0 - (len0 - 1);
                ly -= lastgap;
        }
```

FIGURE 4H

```
/*
 * trace back the best path, count matches
 */
static
getmat(lx, ly, firstgap, lastgap)                                    getmat
        int     lx, ly;                 /* "core" (minus endgaps) */
        int     firstgap, lastgap;      /* leading trailing overlap */
{
        int             nm, i0, i1, siz0, siz1;
        char            outx[32];
        double          pct;
        register        n0, n1;
        register char   *p0, *p1;

        /* get total matches, score
         */
        i0 = i1 = siz0 = siz1 = 0;
        p0 = seqx[0] + pp[1].spc;
        p1 = seqx[1] + pp[0].spc;
        n0 = pp[1].spc + 1;
        n1 = pp[0].spc + 1;

        nm = 0;
        while ( *p0 && *p1 ) {
                if (siz0) {
                        p1++;
                        n1++;
                        siz0--;
                }
                else if (siz1) {
                        p0++;
                        n0++;
                        siz1--;
                }
                else {
                        if (xbm[*p0-'A']&xbm[*p1-'A'])
                                nm++;
                        if (n0++ == pp[0].x[i0])
                                siz0 = pp[0].n[i0++];
                        if (n1++ == pp[1].x[i1])
                                siz1 = pp[1].n[i1++];
                        p0++;
                        p1++;
                }
        }

        /* pct homology:
         * if penalizing endgaps, base is the shorter seq
         * else, knock off overhangs and take shorter core
         */
        if (endgaps)
                lx = (len0 < len1)? len0 : len1;
        else
                lx = (lx < ly)? lx : ly;
```

FIGURE 4I

...getmat

```
        fprintf(fx, "< gaps in first sequence: %d", gapx);
        if (gapx) {
                (void) sprintf(outx, " (%d %s%s)",
                        ngapx, (dna)? "base": "residue", (ngapx == 1)? "": "s");
                fprintf(fx,"%s", outx);
        }
        fprintf(fx, ", gaps in second sequence: %d", gapy);
        if (gapy) {
                (void) sprintf(outx, " (%d %s%s)",
                        ngapy, (dna)? "base": "residue", (ngapy == 1)? "": "s");
                fprintf(fx,"%s", outx);
        }
        if (dna)
                fprintf(fx,
                "\n< score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
                smax, DMAT, DMIS, DINS0, DINS1);
        else
                fprintf(fx,
                "\n< score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
                smax, PINS0, PINS1);
        if (endgaps)
                fprintf(fx,
                "< endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
                firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
                lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
        else
                fprintf(fx, "< endgaps not penalized\n");
}

static  nn;             /* matches in core -- for checking */
static  lmax;           /* lengths of stripped file names */
static  ij[2];          /* jmp index for a path */
static  nc[2];          /* number at start of current line */
static  ni[2];          /* current elem number -- for gapping */
static  siz[2];
static char *ps[2];     /* ptr to current element */
static char *po[2];     /* ptr to next output char slot */
static char out[2][P_LINE];     /* output line */
static char star[P_LINE];       /* set by stars() */

/*
 * print alignment of described in struct path pp[]
 */
static
```

pr_align

```
pr_align()
{
        int     nn;     /* char count */
        int     more;
        register i;

        for (i = 0, lmax = 0; i < 2; i++) {
                nn = stripname(namex[i]);
                if (nn > lmax)
                        lmax = nn;
                nc[i] = 1;
                ni[i] = 1;
                siz[i] = ij[i] = 0;
                ps[i] = seqx[i];
                po[i] = out[i];
        }
```

FIGURE 4J


```
        for (nn = nn = 0; more = 1; more; ) {
                for (i = more = 0; i < 2; i++) {
                        /*
                         * do we have more of this sequence?
                         */
                        if (!*ps[i])
                                continue;

                        more++;

                        if (pp[i].spc) {        /* leading space */
                                *po[i]++ = ' ';
                                pp[i].spc--;
                        }
                        else if (siz[i]) {      /* in a gap */
                                *po[i]++ = '-';
                                siz[i]--;
                        }
                        else {                  /* we're putting a seq element
                                                 */
                                *po[i] = *ps[i];
                                if (islower(*ps[i]))
                                        *ps[i] = toupper(*ps[i]);
                                po[i]++;
                                ps[i]++;

                                /*
                                 * are we at next gap for this seq?
                                 */
                                if (m[i] == pp[i].x[pp[i]]) {
                                        /*
                                         * we need to merge all gaps
                                         * at this location
                                         */
                                        siz[i] = pp[i].n[pp[i]++];
                                        while (m[i] == pp[i].x[pp[i]])
                                                siz[i] += pp[i].n[pp[i]++];
                                }
                                m[i]++;
                        }
                }
                if (++nn == olen || !more && nn) {
                        dumpblock();
                        for (i = 0; i < 2; i++)
                                po[i] = out[i];
                        nn = 0;
                }
        }
}

/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
```

```
dumpblock()
{
        for (i = 0; i < 2; i++)
                *po[i]++ = '\0';
```

FIGURE 4K

...dumpblock

```
        (void) putc('\n', fx);
        for (i = 0; i < 2; i++) {
                if (*out[i] && (*out[i] != ' ' || *(pc[i]) != ' ')) {
                        if (i == 0)
                                nums(i);
                        if (i == 0 && *out[1])
                                stars();
                        putline(i);
                        if (i == 0 && *out[1])
                                fprintf(fx, star);
                        if (i == 1)
                                nums(0);
                }
        }
}

/*
 * put out a number line: dumpblock()
 */
static
nums(ix)                                                        nums
        int     ix;     /* index in out[] holding seq line */
{
        char            nline[P_LINE];
        register        i, j;
        register char   *pn, *px, *py;

        for (pn = nline, i = 0; i < lmax + P_SPC; i++, pn++)
                *pn = ' ';
        for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
                if (*py == ' ' || *py == '\t')
                        *pn = ' ';
                else {
                        if (i%10 == 0 || (i == 1 && nc[ix] > 1)) {
                                j = (i < 0)? -i : i;
                                for (px = pn; j; j /= 10, px--)
                                        *px = j%10 + '0';
                                if (i < 0)
                                        *px = '-';
                        }
                        else
                                *pn = ' ';
                        i++;
                }
        }
        *pn = '\0';
        nc[ix] = i;
        for (pn = nline; *pn; pn++)
                (void) putc(*pn, fx);
        (void) putc('\n', fx);
}


putline(i)                                                      putline
        int     ix;
{
```

FIGURE 4L

...putline

```
        int             i;
        register char   *px;

        for (px = namex[fx], i = 0; *px && *px != ' '; px++, i++)
                (void) putc(*px, fx);
        for (; i < lmax+P_SPC; i++)
                (void) putc(' ', fx);

        /* these count from 1:
         * m[] is current element (from 1)
         * nc[] is number at start of current line
         */
        for (px = out[fx]; *px; px++)
                (void) putc(*px&0x7F, fx);
        (void) putc('\n', fx);
}

/*
 * put a line of stars (segs always in out[0], out[1]): dumpblock()
 */
static
stars()                                                         stars
{
        int             i;
        register char   *p0, *p1, cx, *px;

        if (!*out[0] || (*out[0] != ' ' && *(px=out[0]) != ' ') ||
            !*out[1] || (*out[1] != ' ' && *(px=out[1]) != ' '))
                return;
        px = star;
        for (i = lmax+P_SPC; i; i--)
                *px++ = ' ';

        for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
                if (isalpha(*p0) && isalpha(*p1)) {

                        if (xbm[*p0-'A']&xbm[*p1-'A']) {
                                cx = '*';
                                nm++;
                        }
                        else if (dna && day[*p0-'A'][*p1-'A'] > 0)
                                cx = ':';
                        else
                                cx = '.';
                }
                else
                        cx = ' ';
                *px++ = cx;
        }
        *px++ = '\n';
```

FIGURE 4M

```
        /*
         * strip path or prefix from pn, return len of pn - align()
         */
        static
        stripname(pn)                                                    stripname
                char    *pn;    /* file name (may be path) */
        {
                register char   *px, *py;

                py = 0;
                for (px = pn; *px; px++)
                        if (*px == '/')
                                py = px + 1;
                if (py)
                        (void) strcpy(pn, py);
                return(strlen(pn));

        }
```

FIGURE 4N

```
/*
 * cleanup()    cleanup any tmp file
 * getseq()    read in seq, set dna, len, maxlen
 * g_calloc()    calloc() with error checkin
 * readjmps()    get the good jmps, from tmp file if necessary
 * writejmps()    write a filled array of jmps to a tmp file: nw()
 */
#include "nw.h"
#include <sys/file.h>

char    *jname    "/tmp/homgXXXXXX";        /* tmp file for jmps */
FILE    *fj;

int     cleanup();                        /* cleanup tmp file */
long    lseek();

/*
 * remove any tmp file if we blow
 */
cleanup(i)                                                    cleanup
        int     i;
{
        if (fj)
                (void) unlink(jname);
        exit(i);
}

/*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ';', '<', or '>'
 * seq in upper or lower case
 */
char    *
getseq(file, len)                                             getseq
        char    *file;    /* file name */
        int     *len;     /* seq len */
{
        char            line[1024], *pseq;
        register char   *px, *py;
        int             natgc, tlen;
        FILE            *fp;

        if ((fp    fopen(file,"r"))       0) {
                fprintf(stderr,"%s: can't read %s\n", prog, file);
                exit(1);
        }
        tlen    natgc    0;
        while (fgets(line, 1024, fp)) {
                if (*line       ';' || *line       '<' || *line       '>')
                        continue;
                for (px    line; *px !    '\n'; px++)
                        if (isupper(*px) || islower(*px))
                                tlen++;
        }

        pseq[0]    pseq[1]    pseq[2]    pseq[3]    0;
```

FIGURE 4O

...getseq

```
        py = pseq + 4;
        *len = tlen;
        rewind(fp);

        while (fgets(line, 1024, fp)) {
                if (*line == ';' || *line == '>' || *line == '<')
                        continue;
                for (px = line; *px != '\n'; px++) {
                        if (isupper(*px))
                                *py++ = *px;
                        else if (islower(*px))
                                *py++ = toupper(*px);
                        if (index("ATGCU",*(py-1)))
                                natgc++;
                }
        }
        *py++ = '\0';
        *py = '\0';
        (void) fclose(fp);
        dna = natgc > (tlen/3);
        return(pseq+4);
}

char    *
g_calloc(msg, nx, sz)                                   g calloc
        char    *msg;           /* program, calling routine */
        int     nx, sz;         /* number and size of elements */
{
        char            *px, *calloc();

        if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
                if (*msg) {
                        fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
                        exit(1);
                }
        }
        return(px);
}

/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */
readjmps()                                              readjmps
{
        int             fd = -1;
        int             siz, i0, i1;
        register i, j, xx;

        if (fp) {
                (void) fclose(fp);
                if ((fd = open(pname, O_RDONLY, 0)) < 0) {
                        fprintf(stderr, "%s: can't open() %s\n", prog, pname);

                        while (++i <
                        for (j = dx[dmax].jmp + j < 0 && dx[dmax].jp.x[j] >= xx; j++)
```

FIGURE 4P


```
                        if (j > 0 && dx[dmax].offset && fj) {
                                (void) lseek(fd, dx[dmax].offset, 0);
                                (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmpz));
                                (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
                                dx[dmax].jmp = MAXJMP-1;
                        }
                        else
                                break;
                }
                if (j >= JMPS) {
                        fprintf(stderr, "%s: too many gaps in alignment\n", prog);
                        cleanup(1);
                }
                if (j > 0) {
                        siz = dx[dmax].jp.n[j];
                        xx = dx[dmax].jp.x[j];
                        dmax += siz;
                        if (siz < 0) {                  /* gap in second seq */
                                pp[1].n[pp[1].nx] = siz;
                                xx += siz;

                                /* id = xx - yy + len[1]
                                 */
                                pp[1].x[pp[1].nx] = xx - dmax + len[1];
                                gapy++;
                                ngapy = -siz;
        /* ignore MAXGAP when doing endgaps */
                                siz = (siz < -MAXGAP || endgaps)? siz : MAXGAP;
                                nj++;
                        }
                        else if (siz > 0) {  /* gap in first seq */
                                pp[0].n[pp[0].nx] = siz;
                                pp[0].x[pp[0].nx] = xx;
                                gapx++;
                                ngapx += siz;
        /* ignore MAXGAP when doing endgaps */
                                siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
                                nj++;
                        }
                }
                else
                        break;
        }

        /* reverse the order of jmps
         */
        for (j = 0, i0 = pp[0].nx-1; j < pp[0].nx; j++, i0--) {
                n = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = n;
                n = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = n;
        }
        for (j = 0, i1 = pp[1].nx-1; j < i1; j++, i1--) {
                n = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = n;
                n = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = n;
        }

        if (endgaps) {
                for (j = 0; j < 2; j++) {
                        if (pp[j].n[0] < 0) {
                                n = -pp[j].n[0];
                                pp[j].n[0] = 0;
                                pp[j].x[0] += n;
                        }
                        i = pp[j].nx-1;
                        if (pp[j].n[i] < 0) {
                                pp[j].n[i] = 0;
                        }
                }
        }
}
```

FIGURE 4Q

```
/*
 * write a filled jmp struct offset of the prev one (if any): nw()
 */
writejmps(rx)                                                          writejmps
        int     rx;
{
        char    *mktemp();

        if (!fp) {
                if (mktemp(jname) < 0) {
                        fprintf(stderr, "%s: can't mktemp() '%s'\n", prog, jname);
                        cleanup(1);
                }
                if ((fj = fopen(jname, "w")) == 0) {
                        fprintf(stderr, "%s: can't write '%s'\n", prog, jname);
                        exit(1);
                }
        }
        (void) fwrite((char *)&dx[rx].jp, sizeof(struct jmp), 1, fj);
        (void) fwrite((char *)&dx[rx].offset, sizeof(dx[rx].offset), 1, fj);
}
```

FIGURE 5

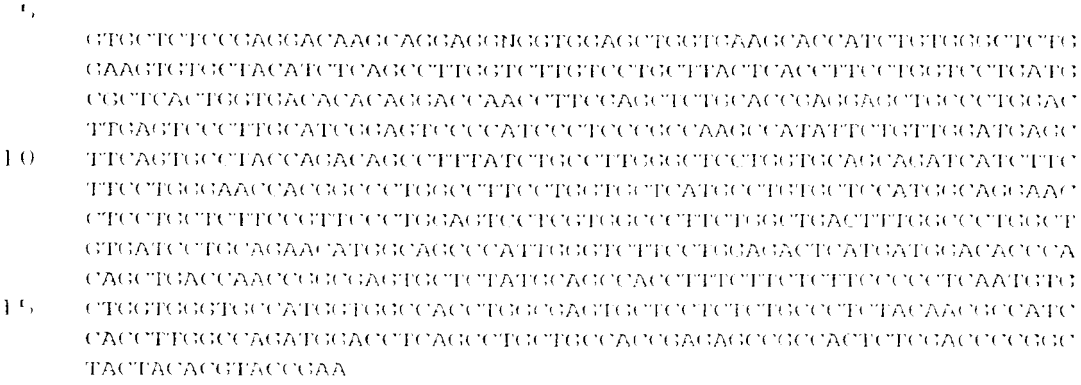


FIGURE 6

FIGURE 7

MSSQPAGNQTSPGATEDYSYGSWYIDEPQGGEELQFPGEVFSCHTSIEPGLYHACLASL
STLVLLLLAMLVERRQLWFDCVRGPFGLFRFRAVPAAVFMVLLSSLCLLLPLEDALPFL
PLASAPSQDPGKTEAPRGAWKTLGLFYYAALYYPLAACATAGHTAAHLLGSTFLSWAHLGV
QVWQRAECPQVFKTYKYYSLLASLPLLLGLGFLSLWYPVQLVPSFSFRTGAGSKGLQSS
YSEETLKHLLCFEKLGSSSYHTTSEHGFLSWAKVCLRHCTYTFQFGFHLLFLKLVLSATLTG
PATYQVALLLLVGVVPTTQKVRAGVTTDVSYLLAGFGIVLSEDEQEVVELVKHHLWALE
VCYISALVLSCLLTFLVLMRSLVTHRTNLRALHRGAALDLSPLHRSFHPSRQAIFCWMS
FSAYQTAFICLGLLVQQLIFFLGTTALAFLVLMPVLHGRNLLLFRSLESSWIFWLTLAL
AVILQHMAAHWVFLETHDGHFQLTNRKVLYAATFLLFPLNVLVGAIVATWRVLLSALYN
AIHLGQMDLSLLPPRAATLDPGYYTYRNFLKIEVSQSHPAMTAFCSLLLQAQSLLPRTM
AAFQDSLRPGEEDEGMQLLQTKDSMAEGARFGASPGPARWGLAYTLLHHFTLQVFRKTA
LLGANGAQP

Important features of the protein:

Signal peptide:
none

Transmembrane domain:
54 71
93 111
140 157
197 214
291 312
356 371
425 444
464 481
505 522

Motif name: N glycosylation site.

8 12

Motif name: N myristoylation site.

50 56
167 173
232 238
308 314
332 338
516 522
618 624
622 628
631 637
652 658

Motif name: Prokaryotic membrane lipoprotein lipid attachment

Motif name: Tissue factor binding site protein as in Swiss

1 17 131

St[illegible]6 Variant Clones

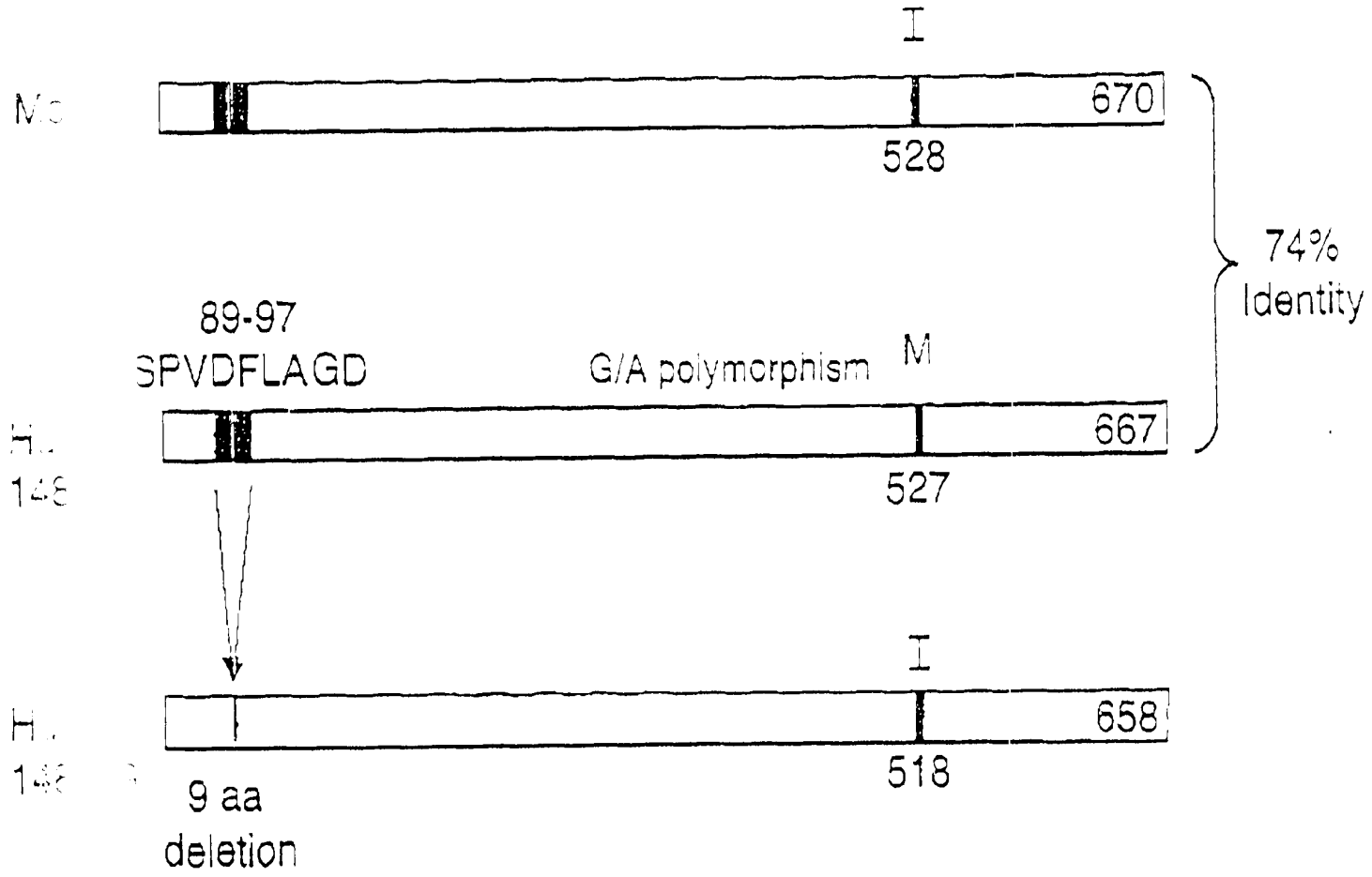
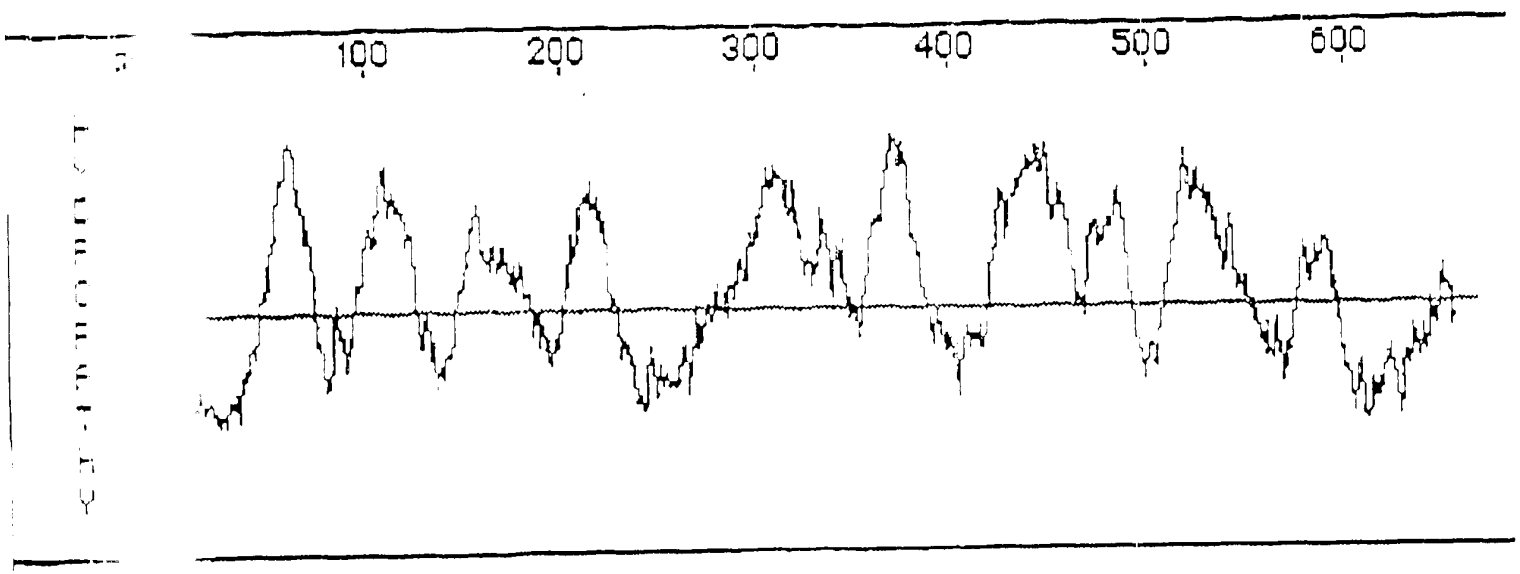


FIGURE 8

Hydrophobicity Plot of Human Stra6



3 kb mRNA
667 Amino Acids -->50% Residues Hydrophobic
73.5 kDa Protein

9 Potential Transmembrane Domains

FIGURE 9

FIGURE 10

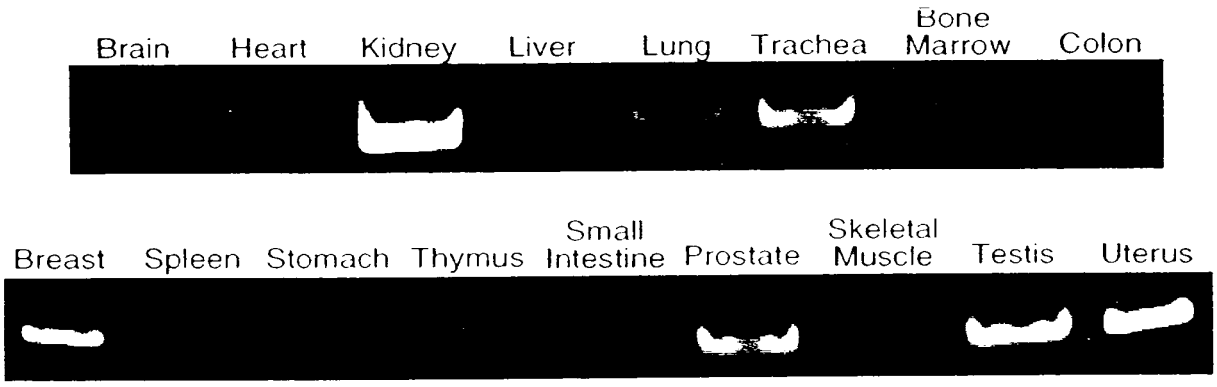

Brain
Heart
Kidney
Liver
Lung
Trachea
Bone Marrow
Colon
Breast
Spleen
Stomach
Thymus
Small Intestine
Prostate
Skeletal Muscle
Testis
Uterus

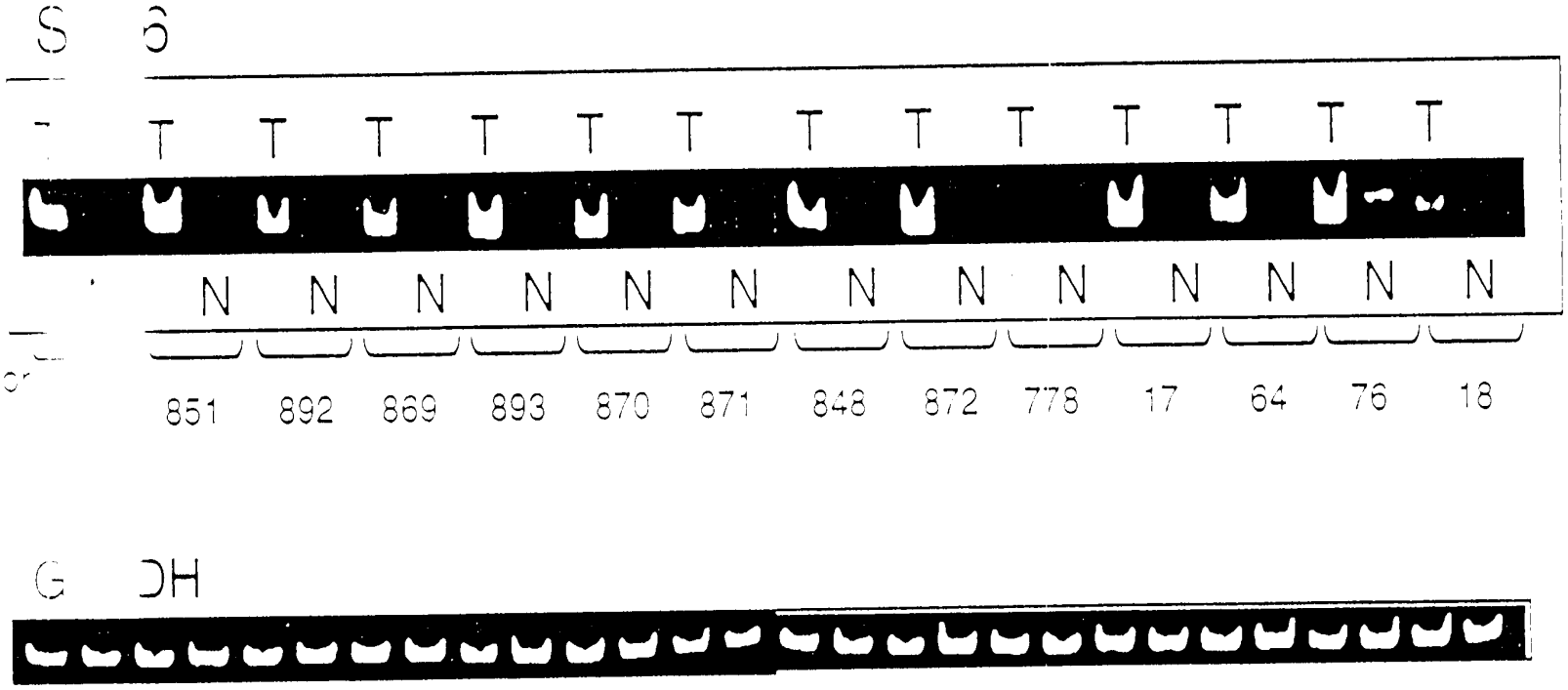
FIGURE 11

Stn RNA Expression in Human Colon Tumor Tissue
Fold Expression Relative to RNA Expression in Normal Mucosa from Same Patient
172
117
850
851
892
869
893
870
871
848
872
778
717
618
664
776
Tumor Sample #

FIGURE 12A

S 6 RNA Expression in Human Colon Tumor Tissue vs Normal Mucosa From the Same Patient

Taqman Product Analysis After 40 Cycles



WJ TM #84 h.Stra6 Primer Set #4 1/4/00

FIGURE 12B

C

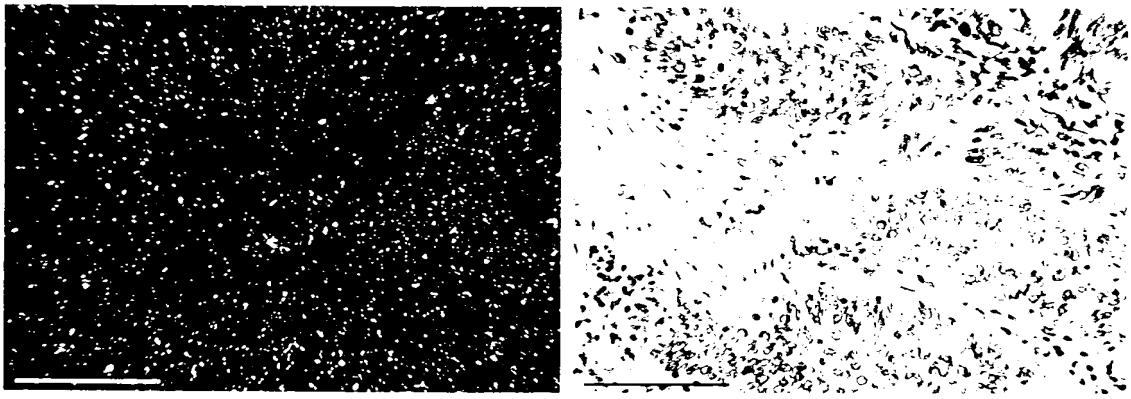
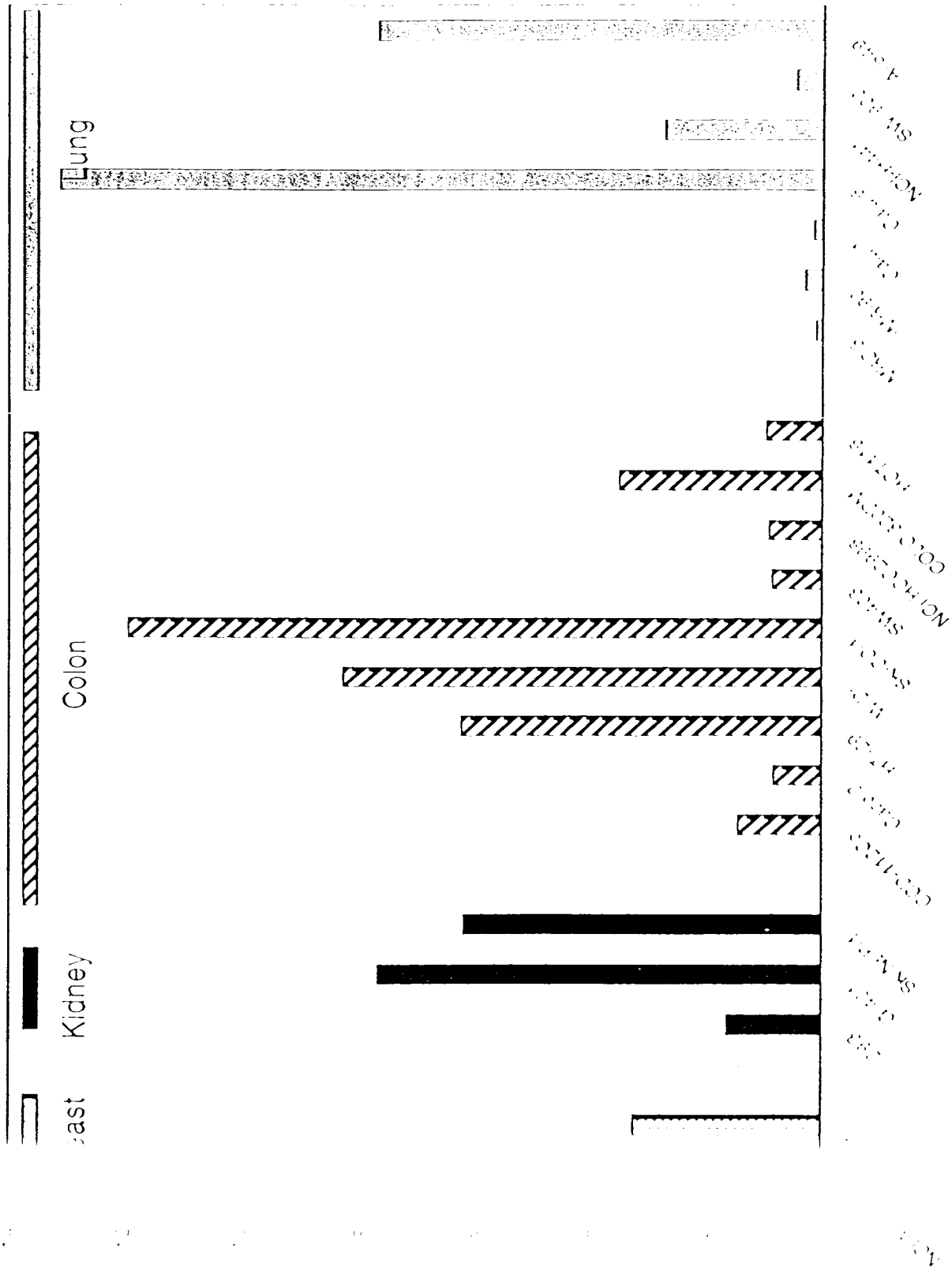
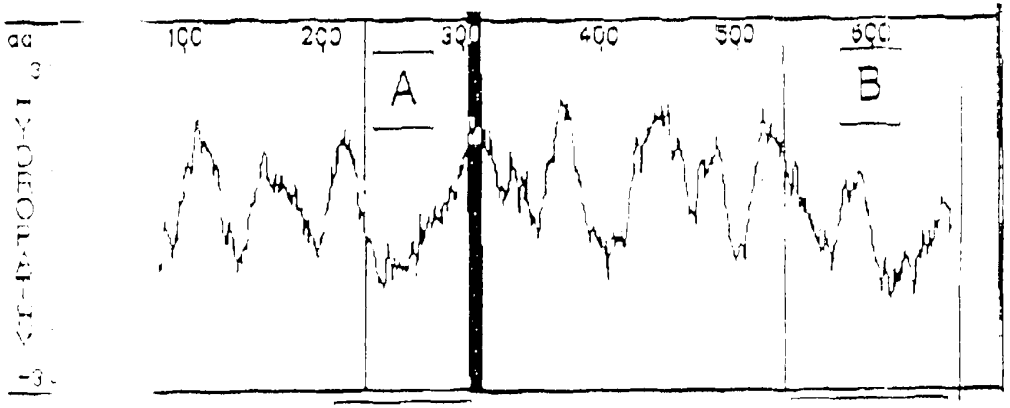


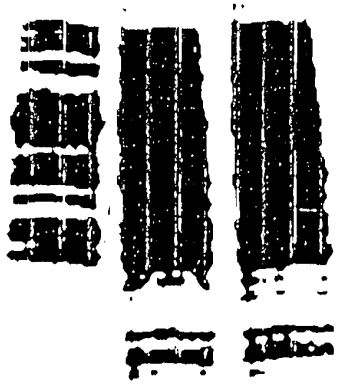
FIGURE 13

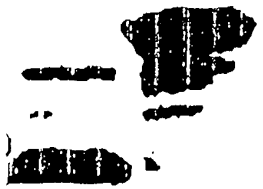


S:[illegible] Peptide Expression in E. coli

Poly-His Cleavable Leader at N-Terminus







pBR322 A B

[illegible] Culture
[illegible]/Lane

mate:
[illegible] µg/ml

[illegible] g/500 ml

[illegible]ansura

FIGURE 14

Stra6 RNA Expression in Human Colon Carcinoma Cells +/- Retinoic Acid

TM#75 (2/28/00)

VD3 = Vitamin D3 (1μM); ATRA = all-trans-retinoic acid (1 μM);
9cRA = 9-cis-retinoic acid (1 μM)

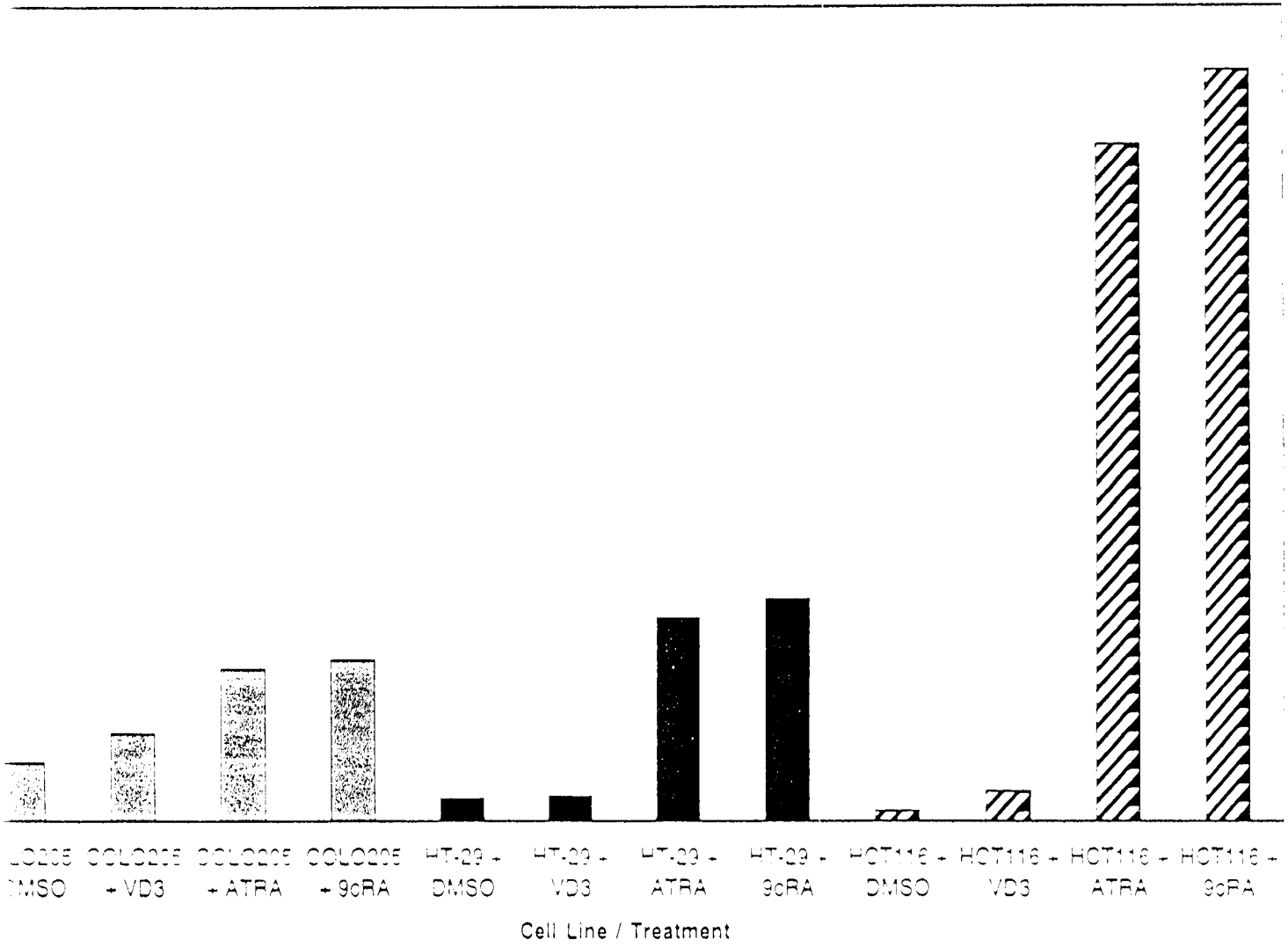


FIGURE 15

FIGURE 16

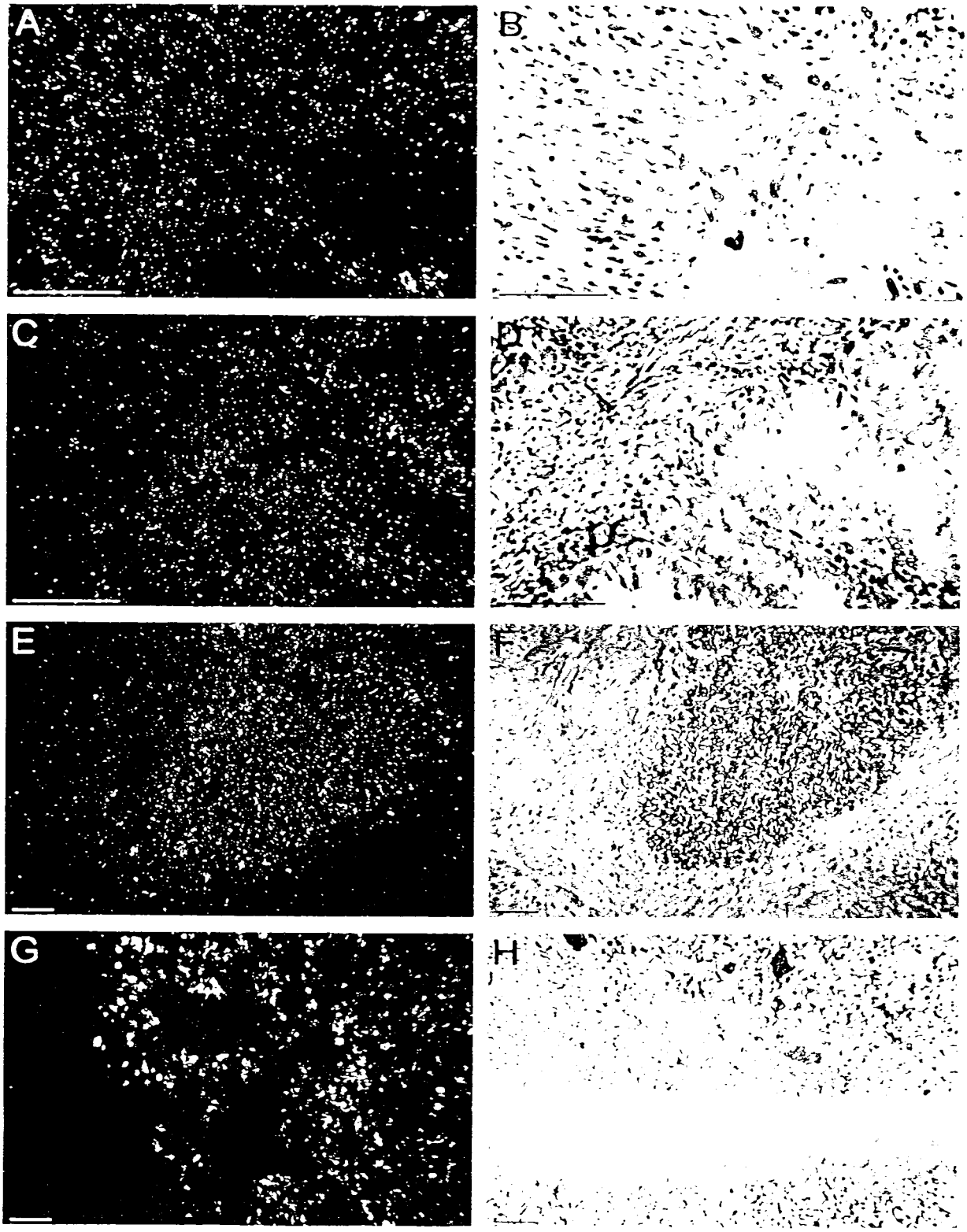


FIGURE 17

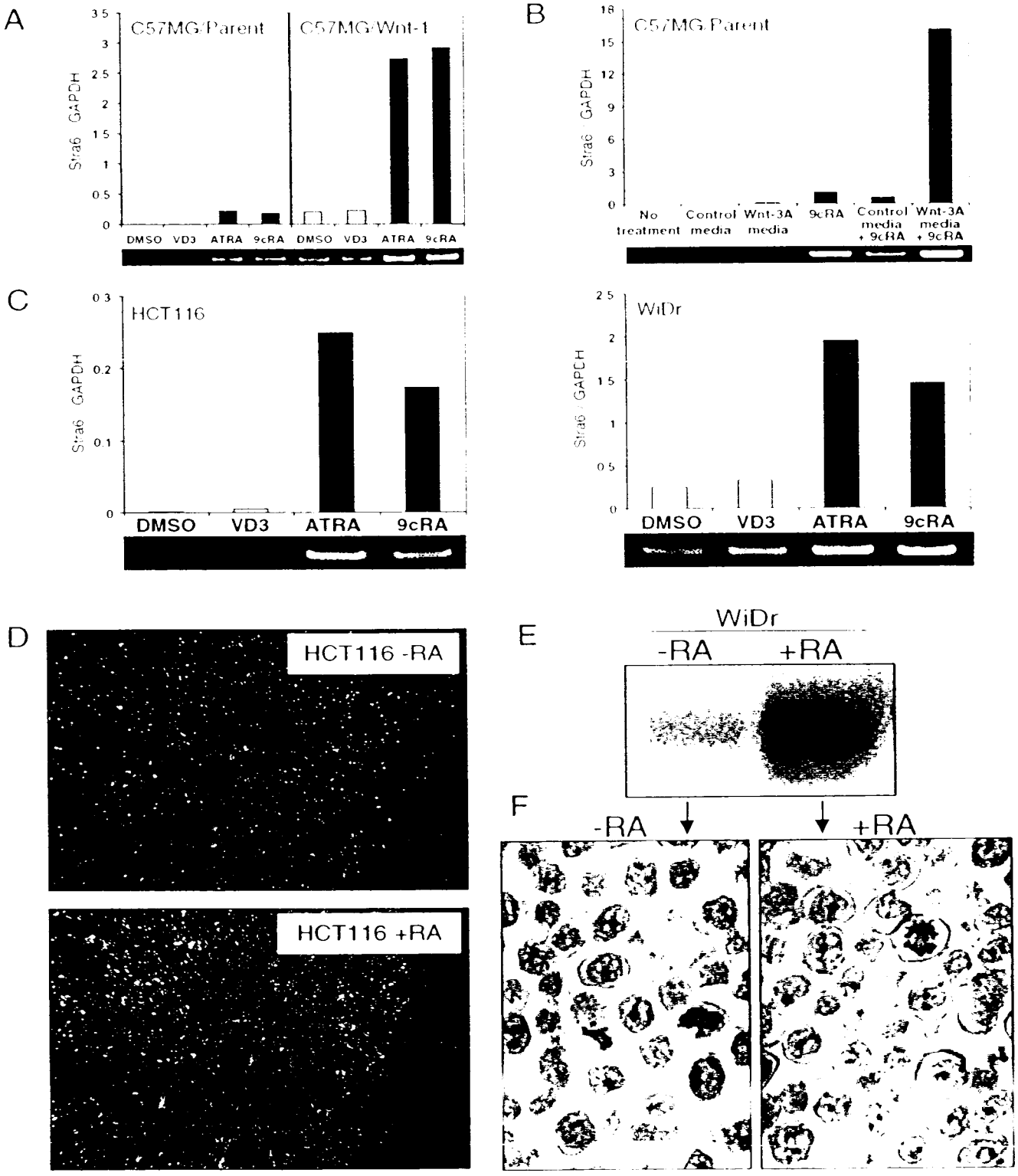
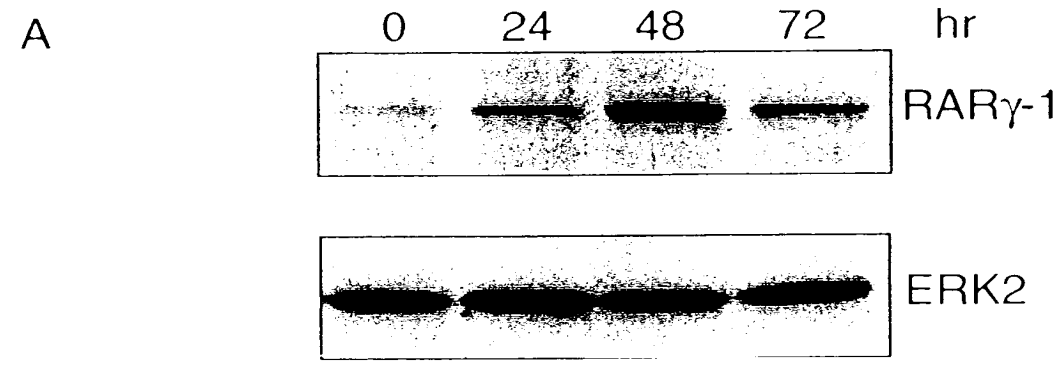
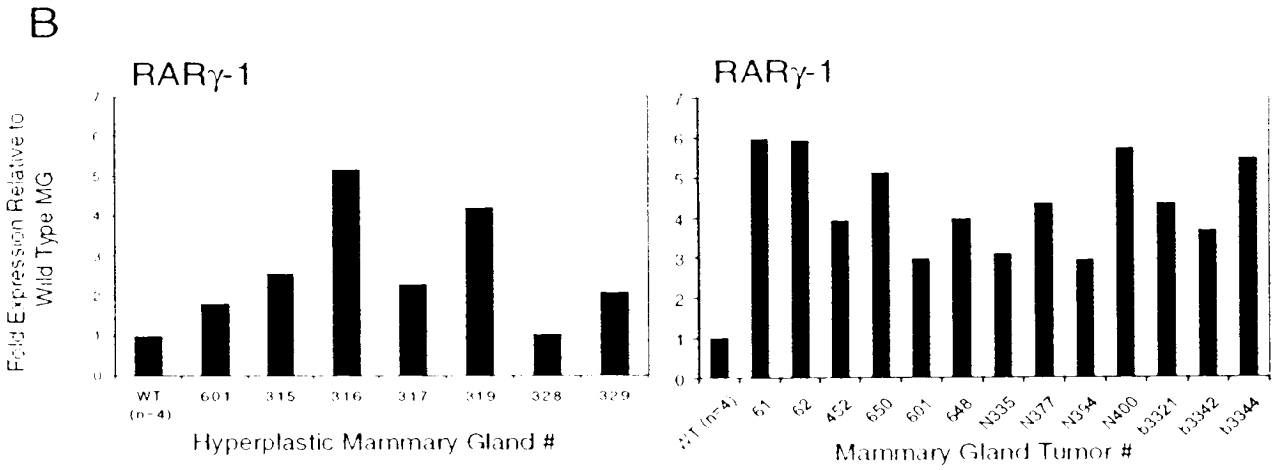

A
C57MG/Parent
C57MG/Wnt-1
Stra6 / GAPDH
DMSO VD3 ATRA 9cRA DMSO VD3 ATRA 9cRA
B
C57MG/Parent
Stra6 / GAPDH
No treatment
Control media
Wnt-3A media
9cRA
Control media + 9cRA
Wnt-3A media + 9cRA
C
HCT116
Stra6 / GAPDH
DMSO VD3 ATRA 9cRA
WiDr
Stra6 / GAPDH
DMSO VD3 ATRA 9cRA
D
HCT116 -RA
HCT116 +RA
E
WiDr
-RA +RA
F
-RA +RA

FIGURE 18


A
0
24
48
72
hr
RARγ-1
ERK2


B
RARγ-1
Fold Expression Relative to Wild Type MG
WT (n=4)
601
315
316
317
319
328
329
Hyperplastic Mammary Gland #
RARγ-1
WT (n=4)
61
62
452
650
601
648
N335
N377
N354
N400
b3321
b3342
b3344
Mammary Gland Tumor #